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Full paper

Oxaliplatin treatment changes the function of sensory nerves in rats

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ABSTRACT

Oxaliplatin (L-OHP) is a platinum-based chemotherapy drug, used in standard treatment of colorectal cancer. L-OHP frequently causes acute peripheral neuropathies. These adverse effects limit cancer therapy with L-OHP. The present study was designed to reveal the changes in sensory nerve function in L-OHP-injected rats. Mechanical static allodynia, dynamic allodynia, and cold allodynia were evaluated using the von Frey test, brush test, and acetone test, respectively. Sensory nerve fiber responsiveness was measured using a Neurometer. The fifth lumbar ventral root was sectioned to record multi-unit efferent discharges. Single intraperitoneal administration of L-OHP induced mechanical static allodynia, dynamic allodynia, and cold allodynia in Wistar/ST rats. The thresholds for paw withdrawal induced by 2000 Hz (Aβ-fiber) and 5 Hz (C-fiber), but not 250 Hz (Aδ-fiber) sine-wave electrical stimulation were reduced in L-OHP-treated rats. Multi-unit efferent discharges were increased by mechanical stimulation using a von Frey filament applied to the plantar surface of the hindpaw. The discharges during and after stimulation were increased in the L-OHP-treated rats. Cold stimulation, but not brush stimulation, increased the discharges in L-OHP-treated rats. These results suggest that sensitization of Aβ- and C-fibers, but not Aδ-fibers, contributes to the development of L-OHP-induced mechanical and cold allodynia.

1. Introduction

Oxaliplatin (L-OHP) is a third-generation platinum agent (1) that binds to DNA and prevents the DNA replication required for mitosis (2). Although it is especially effective in the treatment of colorectal cancer (3), L-OHP causes peripheral neuropathy in many patients (4), which is the most frequent dose-limiting neurotoxicity of L-OHP (5). L-OHP exhibits two types of neurotoxicity (3). One is acute cold-hypersensitivity, which is a characteristic toxicity of L-OHP, and the other is chronic neuropathy, which is common to platinum-containing drugs, caused by the accumulation of platinum in the dorsal root ganglion (DRG) (6). Previous reports have shown an increase in ion-channel (7) and TRPA1 (8) mRNA expression in the DRG after L-OHP treatment. However, the mechanisms underlying L-OHP-induced acute neuropathy still remain unclear. Previous studies have mostly focused on the contribution of sensory receptors; only a few studies have focused on the functional differences in the peripheral nerve. The hypersensitivity induced by L-OHP may result from aberrant changes not only in the sensory reception system but also in the conduction and transduction pain systems.

Primary afferent nerve fibers have been classified into three major classes: unmyelinated C, myelinated thin Aδ, and myelinated Aβ fibers. The sensory C-fibers and Aδ-fibers conduct noxious chemical, mechanical, or thermal stimuli, which in turn cause nociceptive responses. The stimulation of Aβ-fibers is thought to cause mostly innocuous tactile sensations. Therefore, the functions of primary afferent fibers need to be evaluated individually. The Neurometer can selectively activate sensory nerve fibers using sine-wave pulses of different frequencies without affecting nociceptors. It has been reported that frequencies of 5, 250, and 2000 Hz activate C-, Aδ-, and Aβ-fibers, respectively. This stimulus-dependent selective activation of a specific type of primary afferent fiber has been confirmed by electrophysiological (9), pharmacological, and immunohistochemical (10,11) procedures.

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In light of the established importance of the spinal cord in the study of pain mechanisms and analgesics (12,13), several *in vivo* and *in vitro* electrophysiological methods have been developed and used to evaluate spinal cord function in nociceptive transmission. At the spinal level, nociceptive signals are transmitted from the dorsal horn to the motoneurons in the ventral horn, and withdrawal responses are induced through motoneurons to avoid noxious stimuli (14). In a previous study we recorded the discharges from the ventral root induced by mechanical stimulation of the ipsilateral hindpaw plantar surface, and these responses consisted of during- and after-discharges (15). Application of noxious mechanical stimuli to the corresponding hindpaw evoked spinal ventral root after-discharges (6,7). These after-discharges are significantly related to the above-mentioned mechanical hyperalgesia, because these phenomena are prolonged after cessation of the noxious stimuli (5,16). Such after-discharges are also observed in the spinal dorsal horn neurons (16,17) and are simultaneously recorded from motor units after high intensity transcutaneous stimulation (18,19). Since ventral root after-discharges are enhanced in neuropathic pain models, L-OHP treatment might affect ventral root firing after mechanical stimulation.

In this study, we examined the contribution of the various peripheral fibers in the acute phase of L-OHP-induced neuropathy using behavioral and electrophysiological methods. Therapies and prophylaxis for L-OHP-induced neuropathy, such as calcium-magnesium infusions (16,17), pregabalin (18), and Kampo medicines (19), have been reported but the findings are inconclusive (20). It is expected that elucidation of the changes in sensory fibers and the contribution of sensory fibers to neuropathy will be useful for the development of new therapies and prevention of L-OHP neuropathy.

2. Materials and methods

All of the experimental protocols used in the present study were approved by the Animal Care and Use Committee of Nagoya City University, and carried out in accordance with the guidelines of the Japanese Pharmacological Society.

2.1. Behavioral tests

Wistar/ST male rats (6–8 weeks old, SLC, Shizuoka, Japan) were used. Each behavioral test was performed before L-OHP administration (dissolved in 5% glucose, 10 ml/kg i.p.) and 1 and 3 days after administration. Rats were placed in suspended cages with wire-mesh floors. Stimuli were applied alternately three times to the left hindpaw and average scores were generated from the three scores for each rat.

2.2. Assessment of static allodynia

Static allodynia was assessed by measuring hindpaw withdrawal responses to von Frey filaments (Semmes–Weinstein monofilaments, Stoelting, Wood Dale, IL), ranging from 2 to 60 g (2, 4, 6, 8.5, 10, 15, 26, 60 g). The 50% likelihood of a paw withdrawal response (50% threshold) was determined using the up–down method (30). Testing was initiated with the 8.5 g filament, and each filament was applied perpendicular to the plantar surface of the hindpaw with sufficient force to cause a slight bending of the filaments, for about 3 s. In case of a positive response (lifting of the hindpaw), the next weaker filament was used. In case of a negative response (absence of a hindpaw withdrawal response), the next stronger filament was used. This paradigm was continued until four measurements had been obtained after an initial change in behavior, or until four consecutive positive (score of 1.5 g) or five negative scores (score of 80 g) had been obtained. The resulting scores were used to calculate the 50% threshold (31).

2.3. Assessment of dynamic allodynia

Dynamic allodynia was assessed by lightly stroking the plantar surface of the hindpaw with a paintbrush for 3 s. The allodynic response was ranked as follows according to (32): 0, no response or moving the stimulated paw aside; 1, lifting of the stimulated paw toward the abdomen; 2, flinching or licking of the stimulated paw.

2.4. Assessment of cold allodynia

Cold allodynia was assessed with the acetone drop method. A drop (50 μl) of acetone was placed at the lateral side of the plantar hindpaw. Responses evoked by vaporization of acetone were graded with the following 4-point scale (33,34): 0, no response; 1, quick withdrawal, flick, or stamp of the paw; 2, flicking of the hindpaw; 3, repeated flicking of the hindpaw with licking or biting of the lateral side of the hindpaw.

2.5. Electrical stimulation-induced paw withdrawal test

Three days after L-OHP administration rats were gently fixed in hammocks without anesthesia. A pair of ball-shaped electrodes (2 mm in diameter) was fastened to the left plantar surface and instep of the rats. Transcutaneous nerve stimuli using each of the three sine-wave frequencies (5, 250, and 2000 Hz) were applied using the Neurometer CPT/LAB (Neurotron Inc. Baltimore, MD, USA). The minimum intensity (microampere) at which each rat withdrew its paw and/or vocalized was defined as the stimulus threshold. Stimuli were applied at 2 min intervals. Means of three measurements were calculated.

2.6. Measurement of ventral root discharges

After the behavioral study, ventral root discharges were recorded using similar protocols to those reported in our previous studies (15,35). In brief, rats were anesthetized with α-chloralose (150 mg/kg, i.p.), and cannulae were inserted into the trachea for artificial respiration. The spinal cord was transected at the first cervical segmental level. A dorsal laminectomy was performed in the lumbo-sacral region of each rat. Both the ventral and dorsal roots below the sixth lumbar segment were cut distally at their points of exit from the vertebral column. The left fifth lumbar segmental (L5) ventral root was sectioned for recording and the ipsilateral L5 dorsal root was left intact to receive peripheral signals. The entire exposed surgical area was covered with liquid paraffin that was maintained at 36 ± 0.5 °C by radiant heat. Rectal temperature was maintained at 36 ± 0.5 °C.

The left plantar surface of the hindpaw was stimulated using von Frey filaments, paintbrush, and acetone. The ventral root discharges occurring during the 3 s of von Frey filament stimulation were defined as 'during-discharges' and those occurring up to 60 s after the stimulation as 'after-discharges'. The ventral root discharges induced by brush stimuli were evaluated by lightly stroking the plantar surface of the rat's left hindpaw with a paintbrush (brush) for 3 s. The ventral root discharges induced by cold stimuli were evaluated for 30 s after the application of 50 μl acetone onto the plantar surface. These responses were normalized by subtraction of the spontaneous activity measured before application of the stimuli. A pair of Ag–AgCl wire electrodes was used for recording. Motoneuronal multi-unit firing from the left L5 whole ventral root was recorded on a digital recorder (sampling rate: 48 kHz, R-44, Roland, Shizuoka, Japan). The signals were amplified and analyzed

using PowerLab (ADInstruments, Colorado Springs, CO, USA) and Chart software.

2.7. Drugs

α-Chloralose was obtained from Tokyo Kasei (Tokyo, Japan). L-OHP was kindly donated by Tanaka Kikinzoku Kogyo (Tokyo, Japan).

2.8. Statistical analysis

All data are expressed as the mean ± SEM. In the behavioral study, two-tailed non-parametric multiple comparisons with Bonferroni corrections following the Kruskal–Wallis test were used for comparisons between the control and other groups. In the electrophysiological study, t-tests with Bonferroni corrections following one-way analysis of variance (ANOVA) were used for multiple comparisons of the control and other groups (36). Differences at $P < 0.05$ (two-tailed) were considered to be significant.

3. Results

3.1. Effects of L-OHP in the behavioral studies

Intraperitoneal administration of L-OHP at doses of 3 and 6 mg/kg decreased the mechanical threshold 1 day after treatment, and the lower threshold persisted for 3 days (Fig. 1A). In addition, the score of dynamic allodynia in the brush test increased in the 6 mg/kg L-OHP group and became significantly higher 3 days after administration (5% glucose: 0.00 ± 0.00; L-OHP: 0.61 ± 0.20, Fig. 1B). L-OHP at a dose of 3 mg/kg did not show a significant effect in the brush test (Fig. 1B). Cold allodynia evaluated by the acetone test was induced by L-OHP treatment dose-dependently (Fig. 1C). A significant increase in the cold allodynia score was observed 3 days after the administration of L-OHP (6 mg/kg, i.p.) (5% glucose: 0.06 ± 0.06; L-OHP: 1.11 ± 0.31, $P < 0.05$). There were no significant effects on the cold allodynia score at a dose of 3 mg/kg of L-OHP (Fig. 1C).

3.2. Effects of L-OHP on the threshold for electrical stimulation to the hindpaw

Transcutaneous nerve stimulation with each of the three sine-wave frequencies of 5, 250, and 2000 Hz to activate C-, Aδ-, and Aβ-fibers, respectively (9), was applied to the hindpaw of each L-OHP-treated rat. The stimulus threshold for 2000 Hz sine-wave stimuli was significantly and dose-dependently decreased (5% glucose: 602.8 ± 48.0 μA; 3 mg/kg: 433.3 ± 64.1 μA; 6 mg/kg: 338.9 ± 18.1 μA, $P < 0.05$, Fig. 2A). No significant changes in the threshold for the 250 Hz stimuli were observed after L-OHP treatment (5% glucose: 72.2 ± 10.2 μA; 3 mg/kg: 58.7 ± 6.2 μA; 6 mg/kg: 60.6 ± 9.0 μA, Fig. 2B). In response to 5 Hz stimuli, the threshold was significantly decreased in the 6 mg/kg group (5% glucose: 51.9 ± 3.9 μA; 3 mg/kg: 40.3 ± 3.6 μA; 6 mg/kg: 33.3 ± 6.0 μA, $P < 0.05$, Fig. 2C).

3.3. Effects of L-OHP on ventral root discharges

After the Neurometer test, ventral root discharges were recorded under anesthesia. Dose-dependent increases of ventral root discharges occurring during stimulation were induced by von Frey filaments (Fig. 3A) and brush stimuli (Fig. 3C). After-discharges were also slightly but significantly increased (Fig. 3B). We have previously reported that C-fibers contribute to the induction of these after-discharges (15). The ventral root discharges induced by acetone varied greatly and we found no significant differences between L-OHP- and vehicle-treated groups (Fig. 3D).

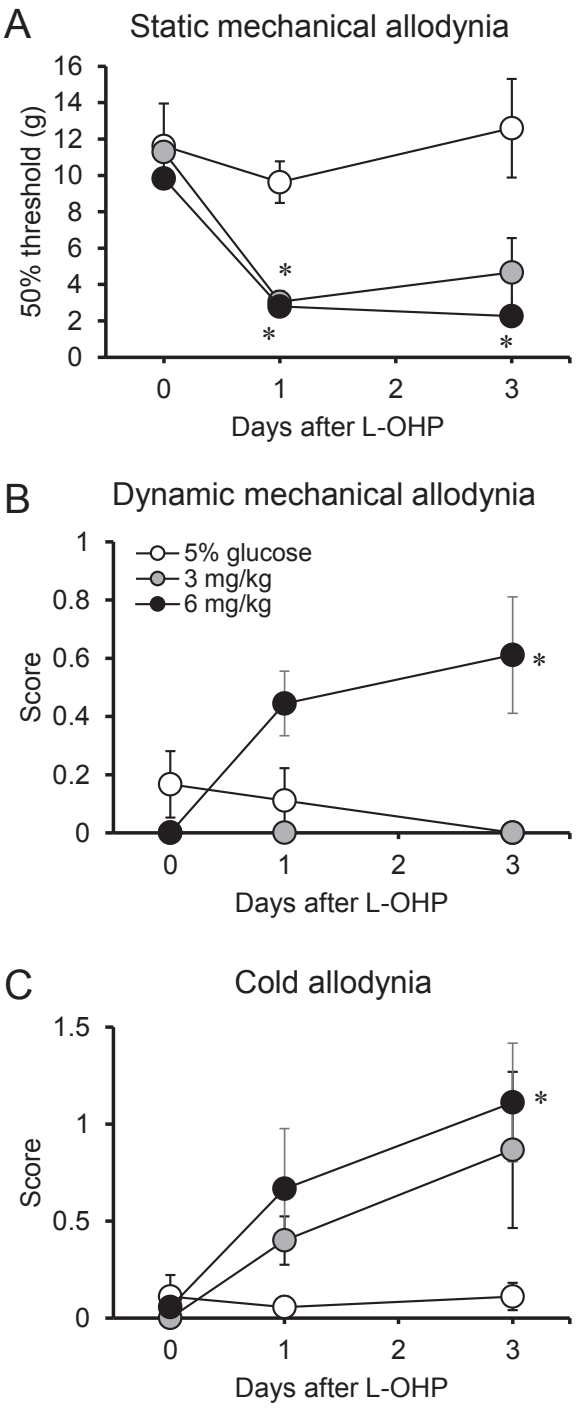

Fig. 1. Time course of oxaliplatin (L-OHP)-induced static mechanical allodynia (A), dynamic mechanical allodynia (B), and cold allodynia (C). L-OHP was administered intraperitoneally. Mechanical threshold, touch, and cold score were assessed by the von Frey test, brush test, and acetone test, respectively. Each point represents the mean ± SEM of five rats. The statistical significance of differences between 5% glucose and the other groups was determined by two-tailed non-parametric multiple comparisons with Bonferroni correction following the Kruskal–Wallis test (two comparisons in three groups). *$P < 0.05$ vs. 5% glucose.

4. Discussion

The present study indicated that acute treatment with L-OHP produced mechanical and cold hyperalgesia in rats (Fig. 1). The withdrawal thresholds in response to an electrical stimulus of

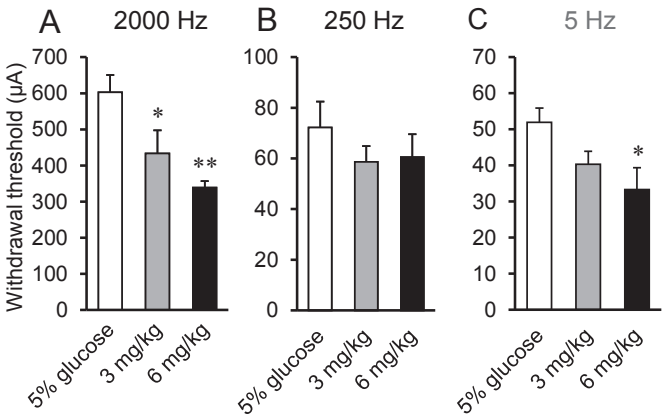

Fig. 2. Paw withdrawal threshold in response to three different sine-wave electrical stimuli in oxaliplatin (L-OHP; 10 mg/kg, i.p.)-treated rats. The current threshold represents the minimum intensity (μA) required to elicit a paw withdrawal response to electrical stimulation with 2000 Hz (A), 250 Hz (B), and 5 Hz (C). The current threshold was assessed by Neurometer. Each point represents the mean ± SEM of five rats. The statistical significance of differences between 5% glucose and the other groups was determined by two-tailed multiple t-tests with Bonferroni corrections following one-way analysis of variance (ANOVA) (two comparisons in three groups). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 5% glucose.

2000 Hz and 5 Hz, but not 250 Hz, were lowered by the L-OHP treatment in the Neurometer test (Fig. 2), suggesting that L-OHP affected the myelinated Aβ- and unmyelinated C-fibers, but not myelinated Aδ-fibers. Moreover, the ventral root after-discharges evoked by mechanical stimulation were increased by L-OHP treatment. These results clearly indicate that L-OHP causes neuropathic pain via sensitization of Aβ- and C-fibers.

A previous report indicated that paclitaxel, a taxane family drug, induced hypersensitization of Aβ- and Aδ-fibers, but not C-fibers (11). The present study clearly showed that L-OHP induced hypersensitization of Aβ- and C-fibers but not Aδ-fibers. The reason for this hypersensitization of different nerve fibers by paclitaxel and L-OHP is not clear but might be due in part to their different modes of action. Although the detailed mechanisms underlying L-OHP- and paclitaxel-induced neuropathic pain remain unknown, the influence of these chemotherapeutic agents might be different for each sensory fiber.

In humans, 2000 Hz (Aβ-fiber) stimuli elicits a touch sensation, 250 Hz (Aδ-fiber) elicits a prick sensation, and 5 Hz (C-fiber) elicits dull and cold pain (21,22). The response threshold is increased by resiniferatoxin or repeated capsaicin treatments at 5 Hz stimulation but not at 2000 or 250 Hz (23). In this study, L-OHP significantly dose-dependently decreased the withdrawal threshold in response to 2000 Hz and 5 Hz stimulation, suggesting that L-OHP sensitizes the Aβ- and C-fibers. This result is consistent with the behavioral study. Mechanical allodynia, especially dynamic mechanical allodynia, is induced by the activation of Aβ-fibers (24). Moreover, since cold sensation is mediated by C-fibers, the sensitization of C-fibers may contribute to the induction of cold allodynia.

We previously developed a method to record the discharges from the ventral root induced by noxious mechanical stimulation of

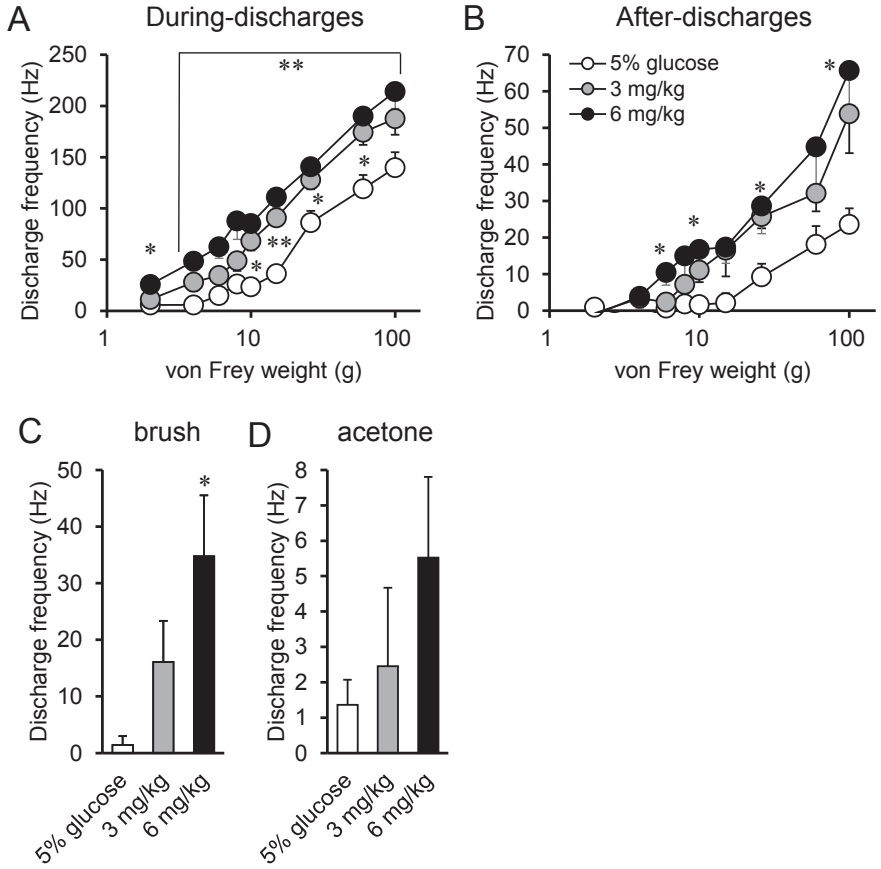

Fig. 3. Ventral root discharges evoked by von Frey filaments, paintbrush, and acetone. Each point represents the mean ± SEM of five rats in each group. The statistical significance of differences between 5% glucose and the other groups was determined by two-tailed multiple t-tests with Bonferroni's corrections following one-way analysis of variance (ANOVA) (two comparisons in three groups). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 5% glucose.

the ipsilateral hindpaw plantar surface (15). The discharges are observed during and after stimulation. Low-intensity mechanical stimulation increased only during discharges, whereas a high-intensity stimulation enhanced both during-discharges and long-lasting after-discharges. Therefore, the magnitude of ventral root discharges may reflect the intensity of the stimulation. In the present study, L-OHP enhanced the frequency of during-discharges induced by von Frey filaments and brush (weaker stimulus than the lowest von Frey filament) stimulation. This increase in the discharge frequency in L-OHP-treated rats suggests the sensitization of spinal nociceptive transmission. Moreover, L-OHP-treated rats showed after-discharges even in response to low mechanical stimuli that did not induce after-discharges in naïve rats. Discharges lasting after stimulation were also observed in the dorsal horn (25–27). The increase in after-discharges was also observed in spared nerve injury model rats (28) and in the spinal dorsal horn of spinal cord injury model rats (29). These results suggest that L-OHP sensitizes nociceptive transmission in the spinal cord, which plays a key role in neuropathic pain via sensitization of Aβ- and C-fibers.

The discharges after acetone application varied greatly in the L-OHP group, but there was a tendency for enhancement relative to the vehicle group. In this electrophysiological study, rats were spinalized and the connection to the upper central nervous system was severed. Licking and biting behaviors evoked by acetone are controlled not only by the spinal cord but also by supraspinal sites. Since the cold allodynia induced by L-OHP might be mediated by both spinal and supraspinal sites, it is possible that even the slight enhancement of the discharges induced by cold stimulation might be related to cold allodynia in L-OHP-treated rats. Moreover, since rats were warmed by electrical heating pads during *in vivo* electrophysiological measurement, it seems likely that the temperature decline by vaporization of acetone might have been reduced. If rats were exposed to a stronger cold stimulation than acetone in our *in vivo* electrophysiological condition, it might be possible to induce strong discharges, including after-discharges, in L-OHP-treated rats.

In conclusion, L-OHP induces mechanical allodynia, dynamic allodynia, and cold allodynia via sensitization of nociceptive transmission in the spinal cord, which may result from the hyper-responsiveness of Aβ- and C-fibers.

Conflict of interest

None declared.

Acknowledgments

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